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Cold acclimation of the Arabidopsis *dgd1* mutant results in recovery from photosystem I-limited photosynthesis☆

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Abstract **We compared the thylakoid membrane composition and photosynthetic properties of non- and cold-acclimated leaves from the *dgd1* mutant (lacking >90% of digalactosyl–diacylglycerol; DGDG) and wild type (WT) *Arabidopsis thaliana*. In contrast to warm grown plants, cold-acclimated *dgd1* leaves recovered pigment-protein pools and photosynthetic function equivalent to WT. Surprisingly, this recovery was not correlated with an increase in DGDG. When returned to warm temperatures the severe *dgd1* mutant phenotype reappeared. We conclude that the relative recovery of photosynthetic activity at 5 °C resulted from a temperature/lipid interaction enabling the stable assembly of PSI complexes in the thylakoid.**

Keywords: Cold acclimation; Digalactosyl–diacylglycerol; Lipid; Monogalactosyl–diacylglycerol; Phosphatidylglycerol; P700; Photosynthesis; Photosystem I; Photosystem II

1. Introduction

The chloroplast thylakoid membrane is perturbed on a regular basis in the field and a high degree of mechanistic plasticity in photosynthetic electron flux is required to avoid photoinactivation and to sustain plant productivity [1]. Acclimation to such environmental change involves modification of the organization and composition of the chloroplast in higher plants [2]. It has been well established that membrane polar lipid composition is one of the important factors controlling the structure and efficiency of thylakoid membranes via specific lipid–protein interactions and/or the dynamic properties of the lipid bilayer [3]. The unique lipid composition of thylakoid membranes is dominated by the two galactolipid headgroups monogalactosyl–diacylglycerol (MGDG) and digalactosyl–diacylglycerol (DGDG) comprising approximately 50% and 20% of the total thylakoid acyl lipid content, respectively. The remaining lipid content is distributed between the negatively charged, phosphatidylglycerol (PG), a sulfolipid sulfoquinovosyldiacylglycerol (SQDG) and some minor phospholipid head groups [3]. The molecular shape of each lipid, determined by both the head group and the fatty acid chains, in part determines whether it forms bilayer or non-bilayer (i.e. H_{II}) structures in mixtures with water. The instability of protein complexes within a thylakoid membrane can therefore be considered in terms of the ratio of non-bilayer forming (e.g. MGDG) to bilayer forming (e.g. DGDG, PG, and SQDG) lipids [4,5]. A high amount of non-bilayer forming lipids are required to form vesicles and membrane fusion [6,7], to form highly ordered protein macroarrays [8] and, due to high curvature stress and/or hydrophobic mismatch in the hydrophobic core of the membrane, to maintain proteins in a functional state [9,10].

Much of the growth temperature-related lipid research has focused on characterizing and manipulating fatty acid desaturase expression in order to control the fatty acid composition of the thylakoid polar lipids. Results have demonstrated that the degree of lipid polyunsaturation increases with reduced growth temperature because low temperatures stimulate desaturase gene expression [11–13]. Arabidopsis mutants defective in specific desaturases show variable sensitivity to low temperature stress relative to wild type. The *fab2* desaturase mutant of Arabidopsis is a dwarf with respect to growth and development at warm temperatures [14], similar to the *dgd1* mutant of Arabidopsis. However, growth of the *fab2* mutant at high temperatures ameliorated the *fab2* phenotype without any change in fatty acid desaturation, indicating that the temperature dependent physical properties of the polar lipids within the thylakoid membrane can directly affect its function. In the *fab2* desaturase mutant, improvement in

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Abbreviations: DGDG, digalactosyl–diacylglycerol; FR, far red light; F_m, maximal chlorophyll florescence in dark-acclimated state; F'_m, maximal chlorophyll florescence in light-acclimated state; F_o, minimal chlorophyll fluorescence in dark-acclimated state; F_s, steady-state fluorescence yield in light-acclimated state; F_v, variable fluorescence ($F_m - F_o$); F_v/F_m, maximal photochemical efficiency of PSII; MGDG, monogalactosyl–diacylglycerol; PG, phosphatidylglycerol; PSI, photosystem I; PSII, photosystem II; SQDG, sulfoquinovosyldiacylglycerol

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growth in the mutant at high temperatures was not accompanied by changed membrane fluidity, suggesting other non-specific effects on the ultrastructure of the thylakoid membranes within the chloroplast [15,16]. To our knowledge, no studies have investigated the low temperature response of plants with a modified lipid head group composition. Cold acclimation involves, amongst other changes, a decrease in lipid bilayer energy status (i.e. a decrease in non-bilayer to bilayer forming head group ratios), a higher degree of lipid desaturation of fatty acids and an increase in total lipid–protein ratios [12,17–21]. Previous studies have demonstrated the correlation of non-bilayer-to-bilayer-forming head group ratios (i.e. MGDG/DGDG) in mediating photosystem activity during cold acclimation [17,20]. Increasing the ratio of MGDG/DGDG increases the minimum temperature at which transition between lamellar phase and gel-to-liquid phase occurs

Fig. 1. Photographs of wild type (left) and *dgd1* mutant (right) of *A. thaliana* during acclimation to 5 °C growth conditions. Numbers define the day of cold acclimation.

Table 1
Mean total daily net photosynthetic CO_2 fixation (A) for warm grown and 42-day-cold acclimated wild type and *dgd1* Arabidopsis plants

Phenotype	Warm grown		Cold acclimated	
Total daily photosynthetic CO_2 assimilation, A, mmol CO_2 m^{-2} day^{-1}				
Temperature (°C)	23	23 → 5	5	5 → 23
Wild type	132.5 ± 9.3	57.1 ± 2.8	159.6 ± 9.1	147.3 ± 5.0
dgd1	84.1 ± 5.1	45.8 ± 4.4	163.0 ± 3.8	108.9 ± 12.2

Measurements were made on plant material at their respective growth temperatures or after a shift to 5 °C (23 → 5) or 23 °C (5 → 23) for 2 days. All plants were maintained with their normal 8 h photoperiod. Each value is the mean ± S.E. of 3–6 plants from one to two different experiments. Each value is corrected for soil respiration and is expressed on a per leaf area basis.

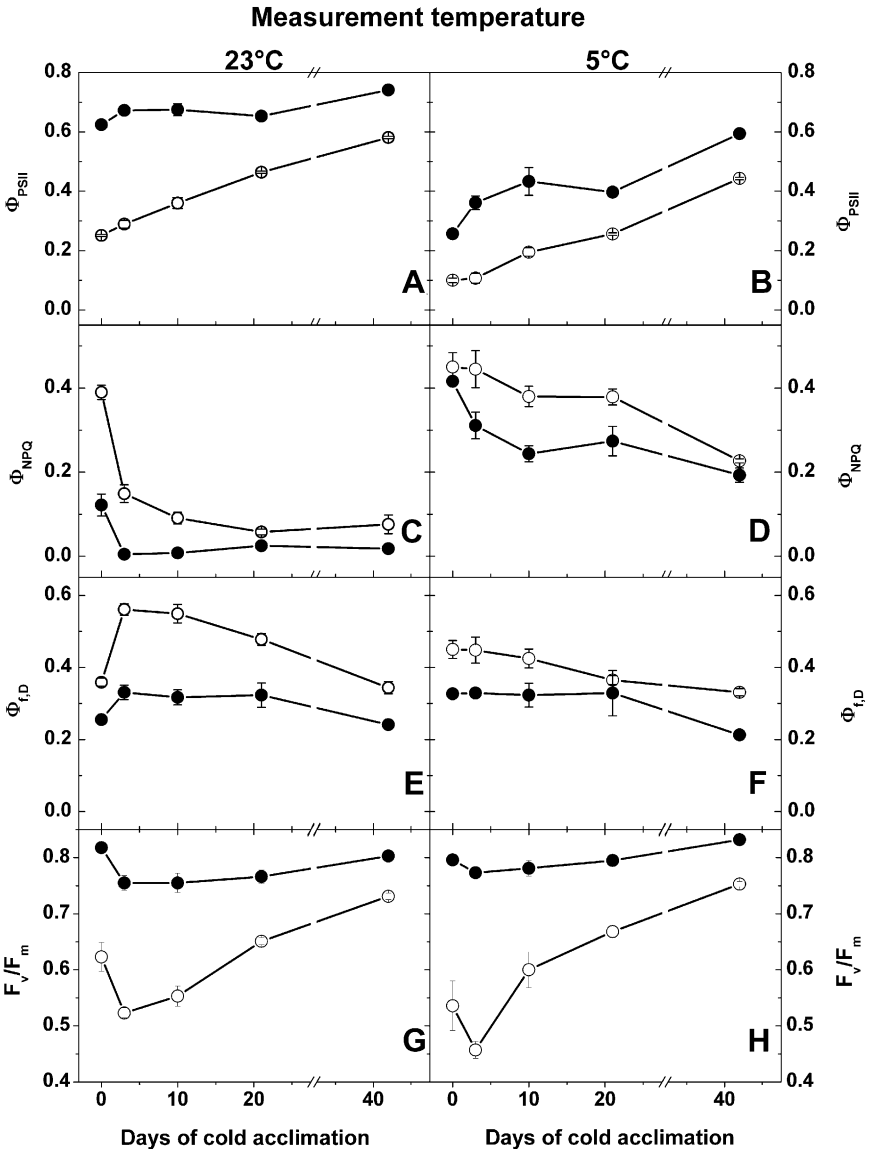

Fig. 2. Fluorescence parameters estimating the effective quantum yields of electron transport, (Φ_{PSII}; A, B), regulated thermal dissipation (Φ_{NPQ}; C, D), constitutive ($\Phi_{f,D}$; E, F) non-photochemical quenching and intrinsic quantum yield of photochemical quenching (F_v/F_m; G, H) for wild type (●) and *dgd1* mutant (○) leaves of *A. thaliana* acclimated for different periods at 5 °C. Plants were dark acclimated for 45 min before being measured at steady-state under 150 μmol quanta m^{-2} s^{-1} at both 23 °C (panels A, C, E, G) or 5 °C (panels B, D, F, H) for no more than 1 h per leaf. Each point is the mean ± S.E. of 4–5 leaves.

[22]. With these observations in mind the ratio of non-bilayer to bilayer forming lipid head groups must be variable enough to maintain thylakoid stability not only during diurnal variations in temperature but also during longer term cold acclimation.

Thylakoid polar lipids, notably DGDG, have been directly implicated in protein import into chloroplasts [23]. Since the isolation and characterization of the *dgd1* mutant of *Arabidopsis thaliana*, lacking 96% of wild type level of DGDG head group lipids [24], a linear relationship has been

established between photosystem II (PSII) function and the DGDG content of thylakoid membranes [25], the underlying structural/functional mechanisms of which have been studied extensively [24,26–29]. Furthermore, recently advances in crystallization of the PSII reaction centre from cyanobacteria have shown that there are up to 14 lipids bound to PSII, including 4 DGDG and 6 MGDG, and that 11 of these lipids appear to form a belt around the PSII reaction centre core, separating it from the antenna [30]. This high lipid composition is suggested to provide the structural flexibility required by the PSII reaction centre during the D1-repair cycle [30], highlighting the importance of the thylakoid lipid composition in PSII reaction centre assembly and function. While less is known of the lipid composition of PSI, studies also utilizing cyanobacteria have shown that there are two lipids, one MGDG and one PG, associated with the psaA and psaB intrinsic core subunits of the reaction center that bind reaction center cofactors [31]. Fyfe et al. [32] have suggested that these two lipid head groups may have specific functional roles in catalyzing the two electron transfer pathways from the reaction center P_{700} to the iron sulfur F_x centers. Two recent studies have also shown that the Arabidopsis *dgd1* mutant has impaired structural and functional stability of photosystem I (PSI) [33,34], contributing to a much higher sensitivity of thylakoid electron flux to photoinactivation of both photosystems in the *dgd1* mutant [34]. Thus the *dgd1* mutant, with artificially increased MGDG/(DGDG + PG + SQDG) and MGDG/DGDG ratios [24], could provide a unique insight into the role of these lipid head group ratios in modulating photosynthetic acclimation to low temperature. The aims of this study were to investigate the long-term cold acclimation responses of Arabidopsis plants with respect to lipid head group composition and to determine whether the *dgd1* mutant, with reduced DGDG content and thylakoid stability, showed an altered capacity to grow and develop at low temperature.

2. Results

2.1. Photosynthetic capacity

Measurements of mean total daily photosynthetic CO_2 assimilation for warm (23 °C) grown and cold (5 °C)-acclimated wild type (WT) and *dgd1* plants show that the small relative size of the warm grown *dgd1* plants (Fig. 1) can be explained, at least in part, by lowered rates of daily net plant photosynthesis (Table 1; $P < 0.01$). The *dgd1* mutant maintained only 63% of the daily net carbon gain of warm grown WT plants (Table 1). Parallel with the smaller plant size and lowered carbon gain was a reduced specific leaf weight of 0.124 ± 0.002 kg m^{-2} in *dgd1* compared that of WT (0.155 ± 0.005 kg m^{-2}). Warm grown plants from both genotypes, when cold stressed by a shift to 5 °C for 2 days, showed reduced photosynthetic activity (Table 1). However, during growth and development at 5 °C, *dgd1* plants fully recovered daily net photosynthesis such that their photosynthetic rates after cold acclimation for 42 days are equivalent to WT (Table 1). There was also a parallel recovery in specific leaf weight for *dgd1* leaves having similar values to WT (0.327 ± 0.012 kg m^{-2} and 0.302 ± 0.013 kg m^{-2} for WT and *dgd1*, respectively).

As demonstrated previously [25,34], the maximum (F_v/F_m) and effective (Φ_{PSII}) quantum efficiencies of PSII were lower in the warm grown *dgd1* plants compared to WT (Fig. 2). Warm grown *dgd1* Φ_{PSII} was reduced by ~40%, and F_v/F_m reduced by ~70% relative to WT (Fig. 2A). This resulted in higher regulated (Φ_{NPQ}) and constitutive (Φ_{fD}) thermal dissipation for the *dgd1* mutant measured at 23 °C (Fig. 2C and E). As indicated by the whole plant gas exchange study, measurements of F_v/F_m (Fig. 2G and H) and Φ_{PSII} (Fig. 2A and B) showed that during cold acclimation *dgd1* plants regained photosynthetic capacity and became similar to WT. Thermal dissipation of absorbed energy also relaxed more rapidly in the *dgd1* plants following cold acclimation, particularly when shifted back to the warmer measuring temperature (Fig. 2C and D), and by day 42 values in the newly-formed rosettes of *dgd1* plants converged with those of WT. The photochemistry of cold acclimated *dgd1* leaves was not affected by a 1-h shift from 5 °C to 25 °C (Fig. 2). However, when the cold acclimated plants were shifted back to 23 °C for 2 d, photosynthesis declined by 74% in the *dgd1* plants compared to WT (Table 1; $P < 0.047$). This suggests that the regression back to the *dgd1* phenotype at 23 °C was not an artifact of measurement temperature but was the result of long-term negative effects on the stability of the thylakoid of the *dgd1* mutant and thus growth at 23 °C.

2.2. PSI photochemistry

The far red (FR) light-induced absorbance decrease at 820 nm (ΔA_{820}) of WT and *dgd1* leaves was used to estimate the photochemistry of PSI from warm grown and cold acclimated leaves [35]. The extent of P700 photooxidation

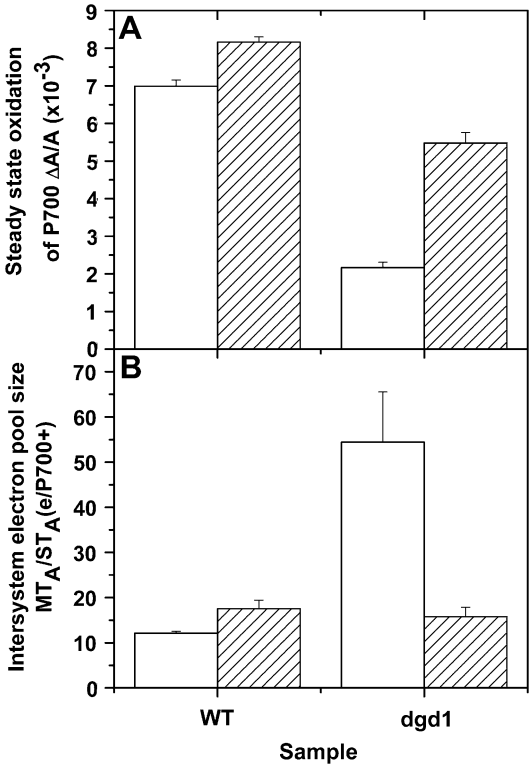

Fig. 3. Steady-state oxidation state of P700 ($\Delta A_{820}/A_{820}$; panel A) and intersystem electron pool size (MT_A/ST_A; panel B) for warm grown (blank columns) and cold acclimated (hatched columns) wild-type and *dgd1* mutant of *A. thaliana*. Each measurement is the mean ± S.E. of 3–6 leaves.

($P700^+$) was measured *in vivo* from FR light induced P700 transients as $\Delta A_{820}/A_{820}$ (Fig. 3A). The apparent electron donor pool size to PSI was assessed by measuring single and multiple turnover flash induced ΔA_{820} under steady-state oxidation of PSI by FR light (Fig. 3B; [36]). In non-acclimated leaves it was clear that the relative amount of $P700^+$ in *dgd1* was only 31% of WT (Fig. 3A) and this was accompanied by a 4.5-fold increase in the intersystem electron pool size in non-acclimated *dgd1* plants compared to WT (Fig. 3B). However following cold acclimation, we observed a significant increase in the relative amount of $P700^+$ in *dgd1* (Fig. 3A) and the size of intersystem electron pool size was reduced in the newly formed rosette of cold acclimated *dgd1* plants to levels similar to WT (Fig. 3B).

2.3. Total pigment, lipid and chloroplast protein analyses

In order to determine whether the loss of thylakoid function in the *dgd1* mutant (and its subsequent recovery) were, in part, due to a loss (and recovery) of structural components, assays of total chloroplast pigments, polar lipids and protein contents were undertaken. The results demonstrated a reduction in all the chloroplast components as partly observed by Ivanov et al. [34]. Measurement over the cold acclimation period confirmed a parallel recovery in *dgd1* plastid protein, lipid and pigment contents relative to WT (Fig. 4). This recovery resulted in a convergence of most parameters measured except lipid/protein and lipid/chlorophyll ratios that surpassed WT in the *dgd1* mutant (Fig. 4).

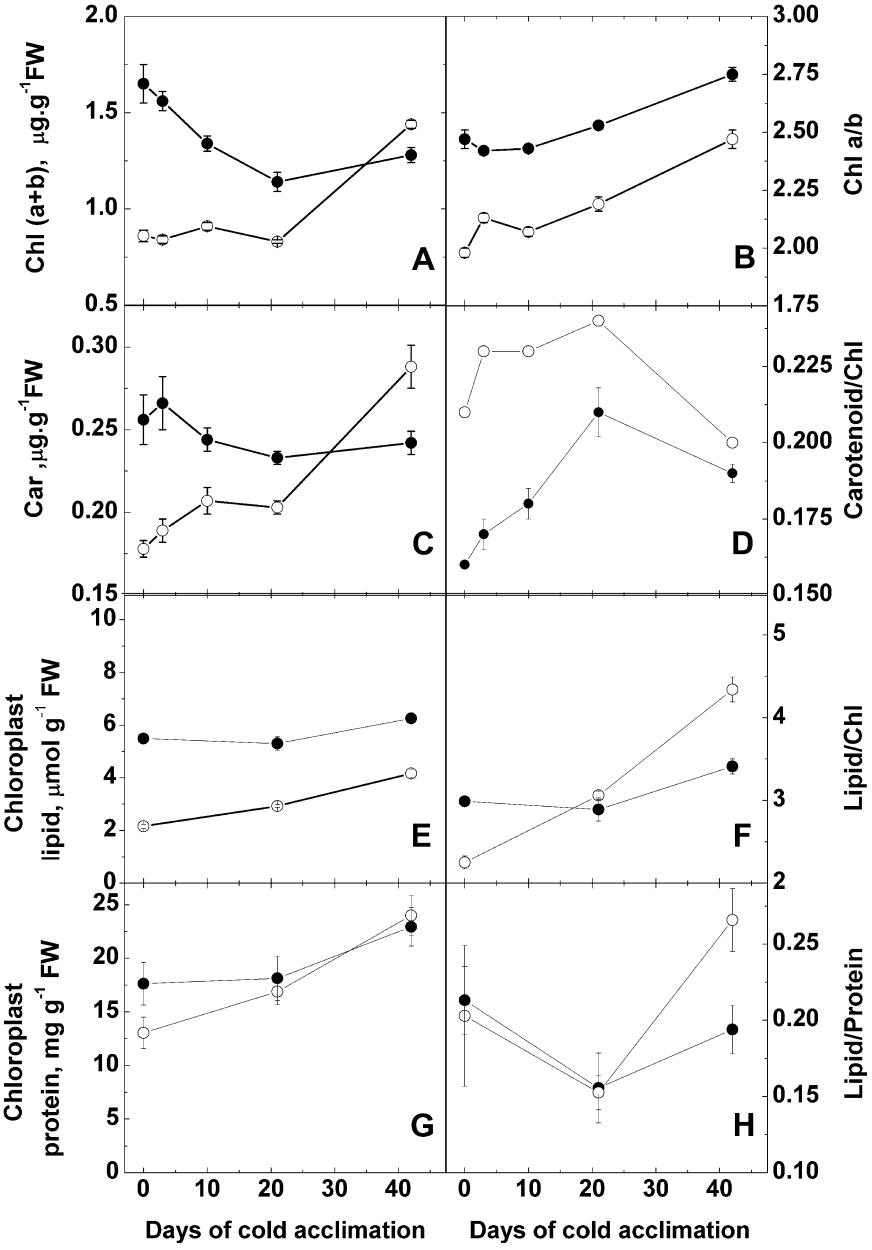

Fig. 4. Pigment, chloroplast protein and lipid composition of wild type (●) and *dgd1* mutant (○) *A. thaliana* plotted against time grown under cold conditions (5 °C). Total chlorophyll (A), carotenoid (C), lipid (E) and membrane protein (G) contents as well as chlorophyll A/B (B), carotenoid/chlorophyll (D), lipid/chlorophyll (F) and lipid/protein ratios (H) are presented. Each point is the mean ± S.E. of 3–4 measurements.

2.4. Lipid composition changes with cold acclimation

As previously shown by Härtel et al. [25], the 23 °C soil-grown *dgd1* plants showed dramatic changes to the lipid composition of those lipids specific to the chloroplast membranes (Fig. 5). Warm grown (23 °C) *dgd1* plants contained less DGDG and more phosphatidylglcerol (PG) than 23 °C soil-grown WT (Fig. 5). This increased the ratio of MGDG/(DGDG + PG + SQDG) from 1.2 to 1.49 and the ratio of MGDG/DGDG from 2.3 to 30.2 for WT and *dgd1*, respectively (Fig. 5). During cold acclimation, WT lost MGDG (−27%) while MGDG in *dgd1* plants was more stable (−9%; Fig. 5A) such that, by day 42 the initially higher MDGD mole fraction in WT was now similar to that of *dgd1*. As expected from previous work, the mole fraction of DGDG in the *dgd1* mutant reduced by 93% compared to WT (Fig. 5B; [25]). However, the recovery of photosynthesis by *dgd1* plants (Table 1; Figs. 2–4) during cold acclimation was not a result of increased DGDG content in the *dgd1* mutant (Fig. 5B) and the mole fraction of DGDG did not change significantly for either the WT and *dgd1* mutant during cold acclimation (Fig. 5B). The molar ratio of DGDG/Chl significantly increased from 0.72 ± 0.05 to 1.21 ± 0.04 in wild type ($P = 0.001$) but this trend was not observed in the *dgd1* mutant, with 0.05 ± 0.01 and 0.034 ± 0.002 ($P = 0.1$) for warm grown and cold acclimated leaves. The mole fraction of PG declined marginally by 13% and 7% for WT and *dgd1*, respectively, although the significantly higher PG fraction was always maintained in *dgd1* (Fig. 5C). The molar ratio of PG/Chl was initially higher in the warm grown *dgd1* mutant compared to wild type with 0.52 ± 0.01 and 0.77 ± 0.03 for wild type and *dgd1*, respectively ($P < 0.001$). However, this ratio increased in cold acclimated leaves to be the same for both genotypes of 0.87 ± 0.03 and 0.89 ± 0.03 for wild type and *dgd1*, respectively ($P = 0.67$). The overall changes during

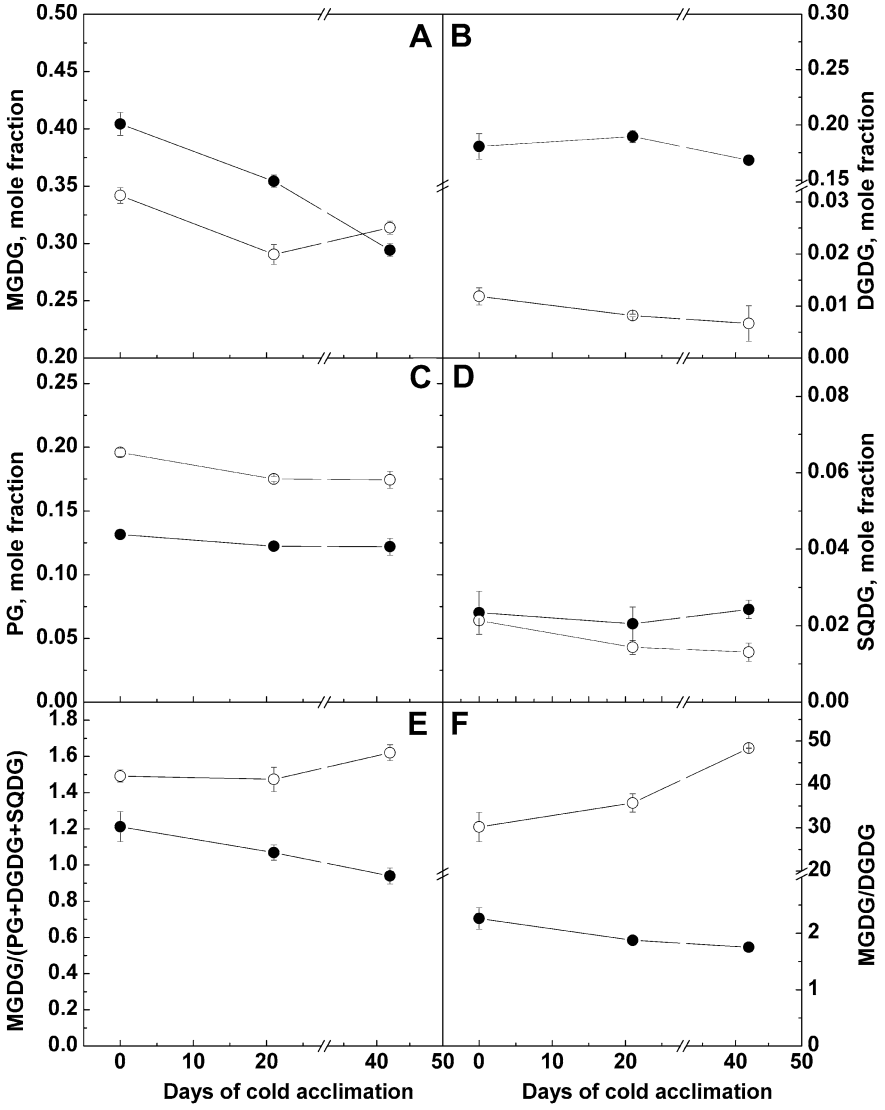

Fig. 5. Polar lipid composition plotted against days of cold acclimation from leaves of wild type (●) and *dgd1* (○) mutant of *A. thaliana*. MGDG, monogalactosyl–diacylglycerol (A); DGDG, digalactosyl–diacylglycerol (B); PG, phosphatidylglycerol (C): SQDG sulfoquinovosyldiacylglycerol (D), MGDG/(PG + DGDG + SQDG) represents the ratio of non-bilayer-to-bilayer-forming lipids in chloroplasts (E) and MGDG/DGDG (F). Values for panels A–D are expressed as a mole fraction of the total lipid content. Each number is the mean ± S.E. of three to four individual determinations. Note the difference *y*-axis scale for each panel.

Table 2
Comparison of fatty acid compositions of lipid classes extracted from leaves of wild type (WT) and *dgd1* mutant of Arabidopsis grown under varying days of cold acclimation at 5 °C

Days of cold acclimation	Genotype	Fatty acid composition							
		16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
MGDG									
0	WT	1.3 ± 0.1	1.8 ± 0.1	2.5 ± 0.2	36.0 ± 0.7			2.5 ± 0.1	55.8 ± 0.5
	dgd1	3.9 ± 0.1	1.5 ± 0.2	3.0 ± 0.2	12.9 ± 0.3			2.4 ± 0.1	76.3 ± 0.4
21	WT	0.6 ± 0.0	0.9 ± 0.1	1.3 ± 0.1	37.7 ± 0.3			0.8 ± 0.1	58.7 ± 0.3
	dgd1	0.9 ± 0.1	1.3 ± 0.2	1.4 ± 0.3	11.5 ± 0.6			0.6 ± 0.0	84.3 ± 0.6
42	WT	0.7 ± 0.2	0.9 ± 0.2	1.3 ± 0.1	39.3 ± 0.3			0.7 ± 0.1	57.1 ± 0.3
	dgd1	0.5 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	19.1 ± 0.3			0.5 ± 0.0	77.9 ± 0.3
DGDG									
0	WT	15.0 ± 0.7	1.2 ± 0.4	1.7 ± 0.3	2.1 ± 1.2	2.6 ± 0.2	2.3 ± 0.2	5.0 ± 0.4	70.2 ± 2.9
	dgd1	20.0 ± 0.3	6.5 ± 0.6	6.2 ± 0.4	5.5 ± 2.0	4.8 ± 0.6	4.3 ± 0.5	2.9 ± 0.5	49.8 ± 4.0
21	WT	11.9 ± 0.3	1.2 ± 0.1	1.4 ± 0.1	0.3 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	79.0 ± 0.3
	dgd1	19.4 ± 1.3	4.3 ± 0.6	9.7 ± 2.9	4.5 ± 0.5	5.0 ± 0.4	4.4 ± 0.3	3.8 ± 0.7	49.0 ± 1.3
42	WT	11.0 ± 0.4	0.9 ± 0.1	0.9 ± 0.1	0.1 ± 0.0	2.6 ± 0.1	2.3 ± 0.0	1.9 ± 0.1	80.2 ± 0.7
	dgd1	19.4 ± 1.8	4.0 ± 1.0	5.9 ± 1.8	5.3 ± 2.3	5.5 ± 0.3	4.8 ± 0.3	3.3 ± 0.3	51.7 ± 4.4
SQDG									
0	WT	35.3 ± 1.3	4.5 ± 0.4			2.5 ± 0.3	5.4 ± 0.5	7.0 ± 0.3	45.3 ± 1.2
	dgd1	40.4 ± 0.7	5.6 ± 0.9			2.8 ± 0.1	4.1 ± 0.2	4.4 ± 0.1	42.8 ± 0.3
21	WT	36.4 ± 4.4	3.5 ± 0.7			1.7 ± 04	3.4 ± 1.0	6.6 ± 2.8	48.3 ± 1.3
	dgd1	35.2 ± 1.9	5.0 ± 0.7			2.7 ± 0.3	3.5 ± 0.4	3.9 ± 0.6	49.7 ± 1.9
42	WT	34.0 ± 1.5	1.7 ± 0.2			1.5 ± 0.1	2.9 ± 0.3	7.2 ± 1.7	52.7 ± 0.9
	dgd1	30.7 ± 3.4	4.6 ± 0.6			2.8 ± 0.6	3.1 ± 0.8	4.9 ± 1.3	53.1± 2.2
PG[a]									
0	WT	28.4 ± 0.5	24.3 ± 0.6			1.0 ± 0.1	6.5 ± 0.3	7.8 ± 0.5	32.1 ± 1.1
	dgd1	26.7 ± 1.0	28.9 ± 1.0			0.8 ± 0.1	5.2 ± 0.5	7.6 ± 0.1	30.8 ± 0.6
21	WT	34.4 ± 0.4	18.5 ± 0.6			0.6 ± 0.1	2.0 ± 0.1	5.5 ± 0.3	38.9 ± 0.3
	dgd1	36.9 ± 0.7	16.2 ± 0.7			0.4 ± 0.1	1.3 ± 0.2	4.6 ± 0.5	40.5 ± 0.4
42	WT	40.7 ± 0.8	10.1 ± 0.5			0.6 ± 0.1	1.9 ± 0.1	6.4 ± 0.3	40.2 ± 0.5
	dgd1	43.2 ± 0.4	9.6 ± 0.4			0.5 ± 0.1	1.4 ± 0.2	5.0 ± 0.4	40.3 ± 1.0

Each value is the mean percentage ± SE of 3–4 samples.
[a]Note that the 16:1 fatty acid reported for PG is the trans-3-hexadecenoic acid.

cold acclimation for the four major lipids of the thylakoid membrane resulted in a significant exacerbation of the difference between the ratios of MGDG/(PG + DG + SQDG) and MGDG/DGDG rather than a convergence as might have been expected from the recovery of photosynthesis in the *dgd1* mutant (Fig. 5).

The fatty acid (FA) compositions of the major lipids of the thylakoid during the course of cold acclimation are shown in Table 2. The major fatty acids in these lipids are palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids. For the most part, the FA compositions were similar between WT and *dgd1* (Table 2). Cold acclimation caused a convergence of mole percentages in the FAs palmitoleic MGDG, linolenic MGDG and a divergence in the FAs palmitoleic DGDG and stearic SQDG between WT and *dgd1* (Table 2). A lowering of the trans-Δ^3-hexadecenoic PG occurred in both genotypes and was more extreme for *dgd1* than WT. The reduction of the ratio of trans-Δ^3-hexadecenoic PG to palmitic PG was consistent with results from cold-grown winter rye [37].

3. Discussion

The results of this study demonstrate that during cold acclimation the *dgd1* mutant was able to recover PSII and PSI photochemistry, photosynthetic CO_2 uptake capacity and thylakoid pigment composition equivalent to WT (Table 1; Figs. 2–5). We also show that this amelioration of the *dgd1* phenotype and recovery in photosynthetic competence was not correlated with an increase in DGDG content in the *dgd1* mutant at the low growth temperature (Table 2). Furthermore, when the low temperature grown *dgd1* rosettes were shifted back to warm conditions, daily CO_2 uptake declined in the *dgd1* mutant over a period of 2 days and the *dgd1* impaired photosynthesis phenotype reappeared. The recovery of the *dgd1* phenotype at low temperature without any change in DGDG content suggests that the effects of reduced thylakoid DGDG content are not specific to the properties of DGDG per se but rather that the global physical properties of the thylakoid membrane are affected by a reduction of DGDG relative to the other major membrane lipids. This 'non-specific' effect is offset by reduced temperature. If the effect of reduced DGDG in the thylakoid of the *dgd1* mutant is non-specific, then earlier conclusions ascribing DGDG as the limiting factor for PSII or PSI structure and function in this *dgd1* mutant under warm growth conditions need to be reinterpreted as a response to the integrated physical properties of the thylakoid membrane [33,34].

It has been suggested that lateral heterogeneity of thylakoid membranes arises from the tendency of LHCII to aggregate in ordered granal stacks and then from steric constraints on the occupancy of appressed membranes by PSI and ATP synthase [38,39]. The vertical lumenal gap in stacked grana varies

between 14 and 21 nm and active shrinking of the lumenal volume may occur during light acclimation [40]. The presence of relatively more MGDG in the thylakoid bilayer in the *dgd1* mutant would allow, at warm temperatures, a greater curvature of the granal end margins thus reducing the area available for PSI assembly in the thylakoid margins. Corroboration can be found in Dörmann et al. [24] where the grana/plastid ratio was significantly higher in *dgd1* than wild type despite similar ratios of appressed/non-appressed thylakoids. This suggests that the tighter curvature of the margins allowed more stacking in the same-sized plastids. If the strongly curved margins of the stacked membranes are protein-free [40], then this hypothesis may still be valid if the tighter margin hairpin turns result in increased steric hindrance of the PSI stromal ridge from nearby curved grana or stromal lamellae. This grana margin curvature hypothesis would explain the acceptor side limitation and greater stromal subunit instability of existing PSI complexes in warm grown *dgd1* (Fig. 3; [33,34]). This grana margin curvature hypothesis can also explain the phenomena of limited state transitions and increased cyclic electron transport around existing PSI as discussed by Ivanov et al. [34]. Higher intrinsic curvature and tighter hairpin turns of the granal margins may also be restricting lumenal volume at warm temperatures, which may have significant effects on the donor-side of PSII [29,40].

In addition to steric hindrance and reduced insertion sites, the potential for hydrophobic mismatch of intrinsic proteins may be greater for warm grown *dgd1* compared to either wild type or cold acclimated *dgd1*. Any positive mismatch between lipid hydrophobic length and protein hydrophobic length that arise from enrichments in MGDG and/or anionic PG can distort the lipid bilayer or the protein sufficient to exclude protein from the lipid bilayer altogether or affect membrane packing enough to affect protein complex stability [41,42]. Positive hydrophobic mismatch can increase local bilayer bending around the proteins potentially exacerbating our proposed steric hindrance/insertion site problem [42]. This leads us to suggest that in our study the reduced insertions sites for, and membrane curvature stress and hydrophobic mismatch of, PSI core proteins such as psaA, B, H and L (all of which are reduced in amounts) are excluded from the margins of the *dgd1* mutant. This model explains the presence of the incomplete PSI complexes in the thylakoid; complexes are synthesized but are unable to be inserted into the granal margins [33].

The above model might explain the specific exclusion of PSI at warm growth temperatures but begs the question of how low temperature ameliorates the *dgd1* phenotype. The overall shape of a lipid is not only defined by acyl chain and lipid head group composition but also by environmental factors such as pH and temperature [10]. At low temperatures lipid molecules are packed more tightly together and the acyl chains are both more highly ordered and more extended corresponding to a thicker bilayer [43]. Thus at lower temperatures the shrinkage of MGDG and other membrane lipids reduces curvature stress and would retard positive hydrophobic mismatch of intrinsic PSI proteins [42]. The curvature of the membrane would therefore be reduced in the *dgd1* mutant, causing the initial recovery of the *dgd1* phenotype after a shift to low temperature, and allowing more lipid to be inserted into newly formed thylakoid membrane that presumably represents the more complete longer-term recovery of the *dgd1* phenotype (and even larger MGDG/(DGDG + PG + SQDG); Fig. 5). The recovery of the oxidation state of $P700^+$ and reduced PSI spill-over to PSII at low growth temperatures (Fig. 3) support the hypothesis that assembly of PSI is improved and that steric hindrance of the stromal ridge of PSI is reduced without an increase in the DGDG content in Arabidopsis leaves. We conclude that low growth temperature itself ameliorates a non-specific effect of reduced DGDG whereby PSI is prevented from assembling or properly functioning due to steric hindrance and/or hydrophobic mismatch in the grana margins.

4. Materials and methods

Seeds of the wild type (WT) of *A. thaliana* ecotype Col-2 and the *dgd1* mutant deficient in one of the major acyl lipids of the thylakoid membrane, DGDG [24], were sown in soil and placed in a controlled environment chamber maintained under 8/16-h day/night cycle (150 μmol photons m^{-2} s^{-1}) at 23/18 °C (day/night) and a relative humidity of 75%. Experiments were performed on 11-week-old plants. For cold acclimation the day/night temperature was changed to 5/5 °C, all the other conditions were unchanged. Measurements on the last date were performed on cold developed leaves. Plants were watered with a high P complete nutrient solution (Blomstra, Cederroth, Sweden) every 3–4 d as required.

Total daily net leaf photosynthesis was measured in warm grown and cold acclimated WT and *dgd1* mutants using a purpose-built whole plant gas exchange system [44]. Prior to measurement all dead leaves and surface soil algae were removed from each pot. In the case of cold acclimated plants only the newly developed rosettes were left on each plant. Eight whole plants were placed in separate water-jacketed chambers, four of which were temperature controlled to a soil temperature of 23 °C and the other four controlled to 5 °C using water baths. Growth conditions were otherwise the same as described above. Each chamber was connected to a single CO_2 analyzer (LI-COR 6262, LI-COR, Lincoln, NE, USA) by means of an electronically controlled circuit pump allowing regular measurement of all eight chambers every 15 min. Pots containing only soil and roots from equivalent aged plants were measured to correct for root and soil respiration at each temperature. Gas exchange was measured for 1–2 days subsequent to which the shoot was harvested for shoot weight and area measurements. Only the previous 24 h of gas exchange measurements were used for calculation of net daily photosynthesis on a shoot area basis.

For pigment analyses, 0.1 g of leaf segment was frozen in liquid nitrogen. The frozen leaf segments were homogenized in 80% acetone buffered with 25 mM HEPES and the homogenate was centrifuged at 800 g for 3 min. The supernatant was used for spectrophotometric estimation (Shimadzu MPS-2000, Shimadzu Europe Ltd., Duisburg, Germany) of chlorophylls and carotenoid content per leaf fresh weight [45].

For lipid analysis, 0.2 g of fresh leaves were heated for 10 min at 80 °C in isopropanol and stored at −20 °C or −80 °C. Before extraction, the isopropanol was evaporated under a stream of nitrogen. The remaining material was homogenized in methanol:0.73% NaCl, 4:1 (v/v), and the lipids were extracted [46]. The individual lipids were isolated by TLC and quantified from their acyl group composition [19], except that the trans-esterification of the fatty acids were performed at 80 °C in 5% H_2SO_4 in dry methanol for 2 h. The integrator was exchanged for an SP4270/4290 integrator (Spectra-Physics, San Jose, CA, USA).

Thylakoid samples were assayed for protein content using a modified Lowry et al. [47] method (Bio-Rad DC Protein Assay, Bio-Rad Laboratories, USA). Leaf material was homogenized in 20 mM HEPES, 0.6 M sorbitol, 75 mM glucose, 10 mM $MgCl_2$, 1 mM EDTA, 10 mM NaCl at pH 7.4, squeezed through two layers of 20 μM miracloth and the spun at $10000 \times g$. The pellet was re-suspended in dH_2O, re-spun at $10000 \times g$ and then pellet re-suspended in the above buffer. All extractions were undertaken on ice or at 4 °C. Standard and samples absorbance change was measured at 750 nm in a spectrophotometer (SpectraMAX 190, Molecular Devices, USA). In all cases total chloroplast lipids, pigments and proteins were expressed on a per leaf fresh weight basis.

In vivo chlorophyll fluorescence was measured using a modulation fluorometer PAM 101–103 (Heinz Walz GmbH, Effeltrich, Germany) from the adaxial side of leaf segments. All measurements were per-

formed after 1 h acclimation to darkness at the temperature at which the plants were grown (23 °C or 5 °C). In both the light and dark acclimated state, the minimal fluorescence intensity was measured by analytic modulated light, the maximal fluorescence intensity by saturating pulses (flash light intensity ~4000 μmol photons m^{-2} s^{-1}) of 0.8 s (1 s for *dgd1* mutant) duration. The maximal photochemical efficiency of PSII photochemistry in the dark acclimated state was evaluated as $F_v/F_m = (F_m - F_o)/F_m$. After 25 min (40 min for *dgd1* mutant) acclimation to white actinic light (150 μmol quanta m^{-2} s^{-1}) and ambient O_2 and CO_2 steady-state was reached. After the F'_m (maximal chlorophyll florescence in light-acclimated state) measurement the actinic light was switched off and FR light was applied for 30 s in order to measure the minimal fluorescence intensity in the light-adapted state (F'_o). Fluorescence parameters were used to calculate Φ_{PSII}, Φ_{NPQ} and $\Phi_{f,D}$ that are quantum efficiencies of photochemistry, regulated thermal dissipation and constitutive thermal dissipation, respectively [48].

The redox state of P700 was determined *in vivo* under ambient O_2 and CO_2 conditions using a PAM-101 modulated fluorometer equipped with a dual wavelength emitter-detector ED-P700DW unit and PAM-102 units as described in detail by Ivanov et al. [35]. Far red light (FR; λ_{max} = 715 nm, 10 W m^{-2}, Schott filter RG 715) was provided by a FL-101 light source. The redox state of P700 was evaluated as the absorbance change around 820 nm ($\Delta A_{820}/A_{820}$) in a custom-designed cuvette. The transient reduction of $P700^+$ signal after application of single (ST) and multiple (MT) turnover flashes of white saturating light was used for estimation of the intersystem electron pool size [35,36].

Sample testing was based on a randomized-block sampling strategy. Statistical comparison of means included independent two-sample *t* tests and one- or two-way analysis of variance using Origin statistical software (OriginPro v7.0220, OriginLab, MA, USA). The number of replicates was 3-6. A *P*-value of less than 0.05 was considered significant.

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References

[1] Cruz, J.A., Avenson, T.J., Kanazawa, A., Takizawa, K., Edwards, G.E. and Kramer, D.M. (2005) Plasticity in light reactions of photosynthesis for energy production and photoprotection. J. Exp. Bot. 56, 395–406.
[2] Walters, R.G. (2005) Towards an understanding of photosynthetic acclimation. J. Exp. Bot. 56, 435–447.
[3] Webb, M.S. and Green, B.R. (1991) Biochemical and biophysical properties of thylakoid acyl lipids. Biochim. Biophys. Acta (Biophys.) 1060, 133–158.
[4] Shipley, G.G., Green, J.P. and Nichols, B.W. (1973) The phase behaviour of monogalactosyl, digalactosyl, and sulphoquinovosyl diglycerides. Biochim. Biophys. Acta (Biophys.) 311, 531–544.
[5] Brentel, I., Selstam, E. and Lindblom, G. (1985) Phase equilibria of mixtures of plant galactolipids. The formation of a bicontinuous cubic phase. Biochim. Biophys. Acta (Biophys.) 812, 816–826.
[6] Selstam, E. and Widell Wigge, A. (1993) Chloroplast lipids and the assembly of membranes in: Pigment-Protein Complexes in Plastids: Synthesis and Assembly (Sundqvist, C. and Ryberg, M., Eds.), pp. 241–277, Academic Press, San Diego.
[7] Rilfors, L. and Lindblom, G. (2002) Regulation of lipid composition in biological membranes - biophysical studies of lipids and lipid synthesizing enzymes. Coll. Surf. B-Biointerf. 26, 112–124.
[8] Garab, G., Lohner, K., Laggner, P. and Farkas, T. (2000) Self-regulation of the lipid content of membranes by non-bilayer lipids: a hypothesis. Trends Plant Sci. 5, 489–494.
[9] de Kruijff, B. (1997) Biomembranes - Lipids beyond the bilayer. Nature 386, 129–130.
[10] Brink-van der Laan, E.V., Killian, J.A. and de Kruijff, B. (2004) Nonbilayer lipids affect peripheral and integral membrane proteins via changes in the lateral pressure profile. Biochim. Biophys. Acta (Biomembr.) 1666, 275–288.
[11] Wada, H., Gombos, Z. and Murata, N. (1990) Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. Nature 347, 200–203.
[12] Gombos, Z., Wada, H. and Murata, N. (1994) The recovery of photosynthesis from low-temperature photoinhibition is accelerated by the unsaturation of membrane-lipids - a mechanism of chilling tolerance. Proc. Natl. Acad. Sci. USA 91, 8787–8791.
[13] Somerville, C. and Browse, J. (1996) Dissecting desaturation: Plants prove advantageous. Trends Cell Biol. 6, 148–153.
[14] Lightner, J., James, D.W., Dooner, H.K. and Browse, J. (1994) Altered body morphology is caused by increased stearate levels in a mutant of Arabidopsis. Plant J. 6, 401–412.
[15] Kunst, L., Browse, J. and Somerville, C. (1989) Altered chloroplast structure and function in a mutant of Arabidopsis deficient in plastid glycerol-3-phosphate acyltransferase activity. Plant Physiol. 90, 846–853.
[16] Kunst, L., Browse, J. and Somerville, C. (1989) Enhanced thermal tolerance in a mutant of Arabidopsis deficient in palmitic acid unsaturation. Plant Physiol. 91, 401–408.
[17] Öquist, G. (1982) Seasonally induced changes in acyl lipids and fatty acids of chloroplast thylakoids of *Pinus sylvestris*. Plant Physiol. 69, 869–875.
[18] Chapman, D.J., De-Felice, J. and Barber, J. (1983) Growth temperature effects on thylakoid membrane lipid and protein content of pea chloroplasts. Plant Physiol. 72, 225–228.
[19] Selstam, E. and Öquist, G. (1985) Effects of frost hardening on the composition of galactolipids and phospholipids occurring during isolation of chloroplast thylakoids from needles of Scots pine. Plant Sci. 42, 41–45.
[20] Vogg, G., Heim, R., Goschy, B., Beck, E. and Hansen, J. (1998) Frost hardening and photosynthetic performance of Scots pine (*Pinus sylvestris* L.). II. Seasonal changes in the fluidity of thylakoid membranes. Planta 204, 201–206.
[21] Hurry, V., Huner, N., Selstam, E., Gardeström, P. and Öquist, G. (1998) Photosynthesis at low growth temperature in: Photosynthesis: A Comprehensive Treatise (Raghavendra, A.S., Ed.), pp. 238–249, Cambridge University Press, Cambridge.
[22] Lindblom, G., Oradd, G., Rilfors, L. and Morein, S. (2002) Regulation of lipid composition in *Acholeplasma laidlawii* and *Escherichia coli* membranes: NMR studies of lipid lateral diffusion at different growth temperatures. Biochemistry 41, 11512–11515.
[23] Chen, L.-J. and Li, H.-M. (1998) A mutant deficient in the plastid lipid DGD is defective in protein import into chloroplasts. Plant J. 16, 33–39.
[24] Dörmann, P., Hoffmann-Benning, S., Balbo, I. and Benning, C. (1995) Isolation and characterization of an Arabidopsis mutant deficient in the thylakoid lipid digalactosyl diacylglycerol. Plant Cell 7, 1801–1810.
[25] Härtel, H., Dörmann, P. and Benning, C. (2001) Galactolipids not associated with the photosynthetic apparatus in phosphate-deprived plants. J. Photochem. Photobiol. B-Biol. 61, 46–51.
[26] Härtel, H., Lokstein, H., Dörmann, P., Grimm, B. and Benning, C. (1997) Changes in the composition of the photosynthetic apparatus in the galactolipid-deficient dgd1 mutant of *Arabidopsis thaliana*. Plant Physiol. 115, 1175–1184.
[27] Härtel, H., Lokstein, H., Dörmann, P., Trethewey, R.N. and Benning, C. (1998) Photosynthetic light utilization and xanthophyll cycle activity in the galactolipid deficient dgd1 mutant of *Arabidopsis thaliana*. Plant Physiol. Biochem. 36, 407–417.
[28] Reifarth, F., Christen, G., Seeliger, A.G., Dörmann, P., Benning, C. and Renger, G. (1997) Modification of the water oxidizing complex in leaves of the dgd1 mutant of *Arabidopsis thaliana* deficient in the galactolipid digalactosyldiacylglycerol. Biochemistry 36, 11769–11776.
[29] Steffen, R., Kelly, A.A., Huyer, J., Dörmann, P. and Renger, G. (2005) Investigations on the reaction pattern of photosystem II in leaves from *Arabidopsis thaliana* wild type plants and mutants with genetically modified lipid content. Biochemistry 44, 3134–3142.
[30] Loll, B., Kern, J., Saenger, W., Zouni, A. and Biesiadka, J. (2005) Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. Nature 438, 1040.
[31] Fromme, P., Jordan, P. and Krauss, N. (2001) Structure of photosystem I. Biochim. Biophys. Acta (Bioenerget.) 1507, 5–31.
[32] Fyfe, P.K., Hughes, A.V., Heathcote, P. and Jones, M.R. (2005) Proteins, chlorophylls and lipids: X-ray analysis of a three-way relationship. Trends Plant Sci. 10, 275–282.

[33] Guo, J.K., Zhang, Z.Z., Bi, Y.R., Yang, W., Xu, Y.N. and Zhang, LX. (2005) Decreased stability of photosystem I in dgd1 mutant of *Arabidopsis thaliana*. FEBS Lett. 579, 3619–3624.
[34] Ivanov, A.G., Hendrickson, L., Krol, M., Selstam, E., Oquist, G., Hurry, V. and Huner, N.P.A. (2006) Digalactosyl–diacylglycerol deficiency impairs the capacity for photosynthetic intersystem electron transport and state transitions in *Arabidopsis thaliana* due to photosystem I acceptor-side limitations. Plant Cell Physiol., doi:10.1093/pcp/pcj089.
[35] Ivanov, A.G., Morgan, R.M., Gray, G.R., Velitchkova, M.Y. and Huner, N.P.A. (1998) Temperature/light dependent development of selective resistance to photoinhibition of photosystem I. FEBS Lett. 430, 288–292.
[36] Asada, K., Heber, U. and Schreiber, U. (1993) Electron flow to the intersystem chain from stromal components and cyclic electron flow in maize chloroplasts, as detected in intact leaves by monitoring redox change of P700 and chlorophyll fluorescence. Plant Cell Physiol. 34, 39–50.
[37] Gray, G.R., Ivanov, A.G., Krol, M., Williams, J.P., Kahn, M.U., Myscich, E.G. and Huner, N.P.A. (2005) Temperature and light modulate the trans-Δ^3-hexadecenoic acid content of phosphatidylglycerol: light-harvesting complex II organization and non-photochemical quenching. Plant Cell Physiol. 46, 1272–1282.
[38] Anderson, J.M. (1999) Insights into the consequences of grana stacking of thylakoid membranes in vascular plants: a personal perspective. Aust. J. Plant Physiol. 26, 625–639.
[39] Allen, J.F. and Forsberg, J. (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci. 6, 317–326.
[40] Dekker, J.P. and Boekema, E.J. (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim. Biophys. Acta (Bioenerget.) 1706, 12–39.
[41] Lee, A.G. (2004) How lipids affect the activities of integral membrane proteins. Biochim. Biophys. Acta (Biomembr.) 1666, 62–87.
[42] Kandasamy, S.K. and Larson, R.G. (2006) Molecular dynamics simulations of model trans-membrane peptides in lipid bilayers: A systematic investigation of hydrophobic mismatch. Biophys. J. 90, 2326–2343.
[43] Gennis, R.B. (1989) The structures and properties of membrane lipids in: Biomembranes: molecular structure and function (Gennis, R.B., Ed.), pp. 40–41, Springer-Verlag, New York.
[44] Lundmark, M., Cavaco, A.M., Trevanion, S. and Hurry, V. (2006) Carbon partitioning and export in transgenic *Arabidopsis thaliana* with altered capacity for sucrose synthesis grown at low temperature: a role for metabolite transporters. Plant Cell Environ., doi:10.1111/j.1365-3040.2006.01543.x.
[45] Lichtenthaler, H.K. (1987) Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. J. Plant Physiol. 131, 101–110.
[46] Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.
[47] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265–275.
[48] Hendrickson, L., Furbank, R.T. and Chow, W.S. (2004) A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. Photosynth. Res. 82, 73–81.